

Research Article

Experimental Investigation of the Properties of Electrospun Nanofibers for Potential Medical Application

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Polymer based nanofibers using ethylene-co-vinyl alcohol (EVOH) were fabricated by electrospinning technology. The nanofibers were studied for potential use as dressing materials for skin wounds treatment. Properties closely related to the clinical requirements for wound dressing were investigated, including the fluid uptake ability (FUA), the water vapour transmission rate (WVTR), the bacteria control ability of nanofibers encapsulated with different antibacterial drugs, and Ag of various concentrations. Nanofibre degradation under different environmental conditions was also studied for the prospect of long term usage. The finding confirms the potential of EVOH nanofibers for wound dressing application, including the superior performance compared to cotton gauze and the strong germ killing capacity when Ag particles are present in the nanofibers.

1. Introduction

Skin wounds and their treatment are significant health problems [1–3]. The dressing material plays a key role in treatment and recovery, providing surface protection, tissue and cell restriction, moisture maintaining, air permeability, bacteriostatic controlling activities, drug delivery, and other functions [4, 5]. Electrospinning, also known as electrostatic spinning, is a popular method to fabricate nonwoven fibers with different shapes and sizes [6, 7]. It is currently the only method to prepare fabric of fibers with diameters down to a few nanometers [8]. Fibrous materials of submicron dimensions are potentially better candidates in skin wound treatment than normal dressing materials such as cotton pads and bandages due to a number of advantageous features, including high porosity ratio, high permeability and mechanical strength, and biocompatibility and biodegradability, among others [9–12]. A number of methods have also been developed to add drugs into the electrospinning

process so the nanofibers obtained become carriers [13] for the additional function of anti-inflammation [14]. EVOH was selected for this study due to its good biocompatibility and proven usage in nanofibers preparation with electrospinning [15–17].

In a previous study [18], we presented a method to fabricate poly (ethylene-co-vinyl alcohol) (EVOH) nanofibers encapsulated with Ag nanoparticle using electrospinning technique. The fibers were fabricated with controlled diameters (59 nm - 3 μ m) by regulating three main parameters, that is, EVOH solution concentration, the electric voltage, and the distance between the injection needle tip (high voltage point) and the fiber collector. The study showed the relationship between the electrospinning fabrication parameters and the properties of the fibers produced, such as the diameter of the nanofibers, the regularity of the nanofiber shape, the uniformity of the diameter, and the average length of the uniform fiber. The mechanical strengths of nanofiber

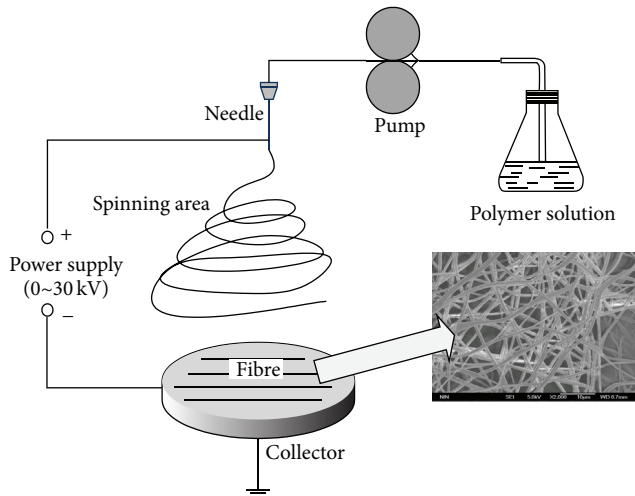
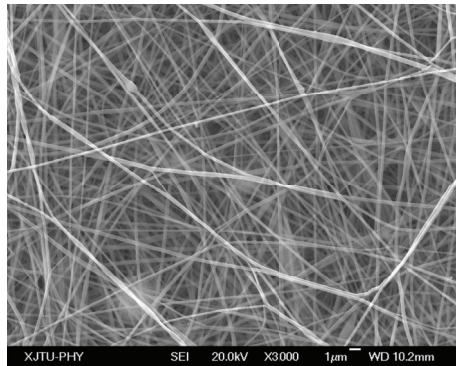
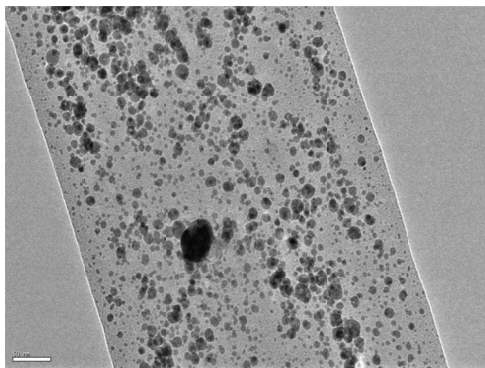


FIGURE 1: The electrospinning system.



(a)



(b)

FIGURE 2: SEM image and TEM image of nanofibers contain Ag: (a) SEM image, (b) TEM image.

mats under monotonic and cyclic tension loading were also discussed [19].

In this study, we investigated the nanofibers' properties closely related to the clinical requirements for wound dressing. The fluid uptake ability, the water vapour transmission rates, the bacteria control ability of nanofibers encapsulated with different antibacterial drugs and Ag of various

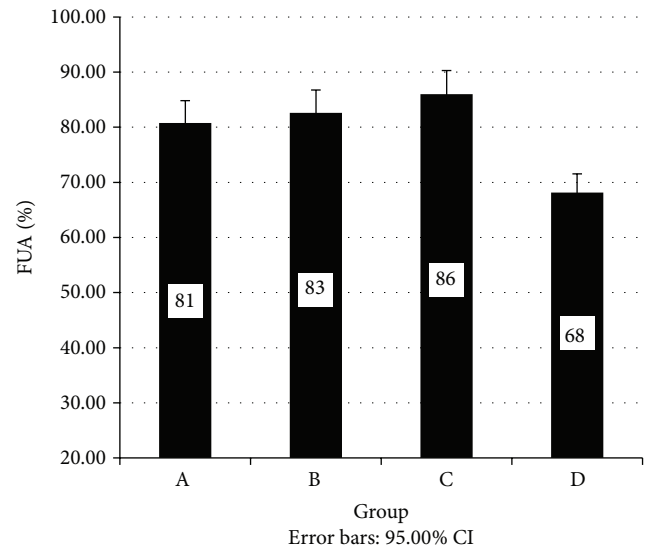


FIGURE 3: The fluid uptake ability of different nanofibers (A, B, and C) and cotton gauze (D).

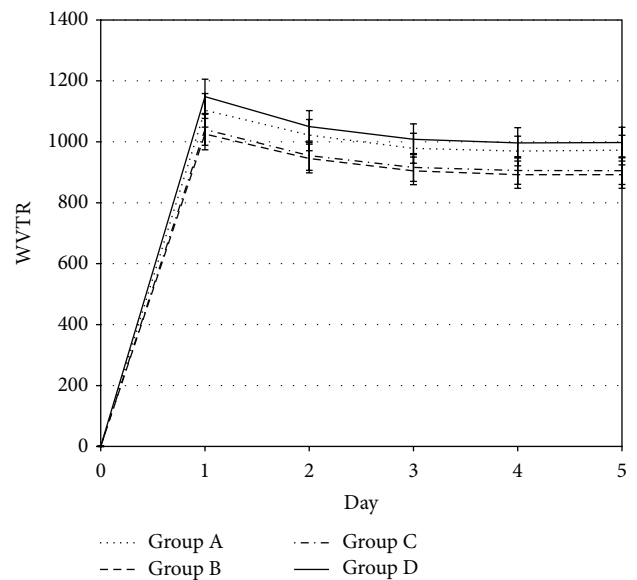


FIGURE 4: Changes of WVTR of nanofiber groups in terms of time.

concentrations, and the degradation of EVOH nanofibers under different environmental conditions were investigated. The study was entirely experiment based and is reported in the following order in this paper. First, the materials used were discussed, followed by a brief introduction on the electrospinning process. The tests and results of fluid up taking, water vapour transmission, and bacteria control were then reported, followed by degradation results. Discussion on the findings and conclusions were then provided in view of the potential medical application.

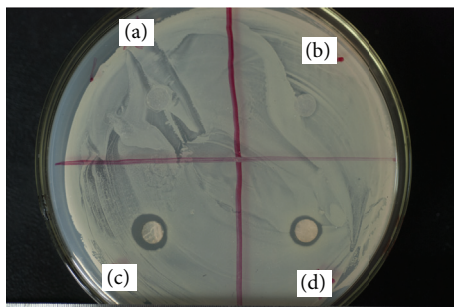


FIGURE 5: Bacteria test after 6 hours of incubation at 37°C: (a) pure EVOH nanofiber and the rest are nanofibers containing (b) gentamicin (60 k units in 10 mL solution), (c) Ag nanoparticle (0.1 g in 10 mL solution, and (d) iodine (0.1 g in 10 mL solution).

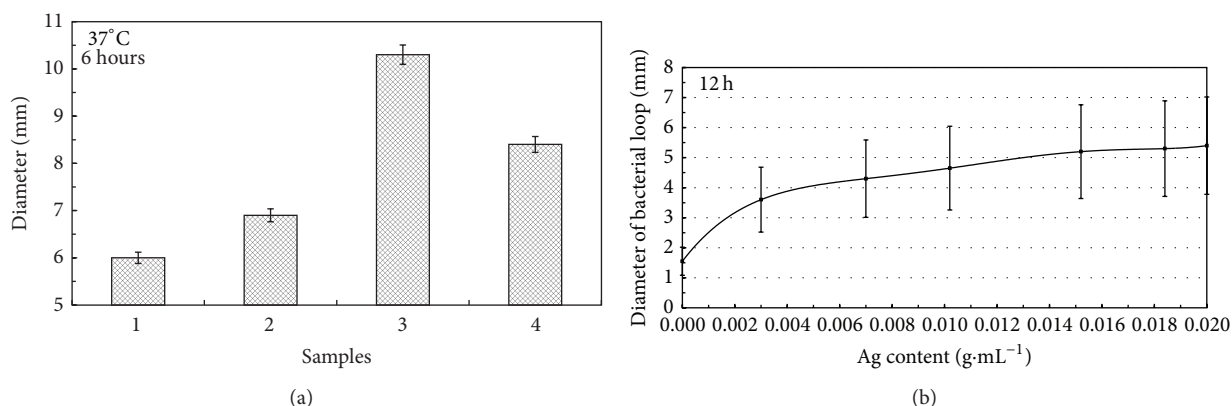


FIGURE 6: (a) Average diameter of bacterial loop, 6 mm, indicates no antibacterial effect: (1) Pure EVOH nanofiber, (2) nanofiber containing gentamicin, (3) nanofiber containing Ag nanoparticle, and (4) nanofibers containing iodine. (b) Averaged bacteriostatic loop diameter plotted as a function of Ag concentration.

2. Material and Electrospinning Process

2.1. Materials. Poly (ethylene-co-vinyl alcohol) (EVOH) was used to make nanofibers in this study. The material was ordered in granular form from Sigma-Aldrich (Batch number: 12822PE). Solid granules were diluted into solutions of 80% 2-propanol/water, in composition of EVOH from 7.5, 10, and 12% (wt%) for injection use.

For the antibiotics used in the study, AgNO₃ was obtained in powder form from Alfa Aesar (Alfa Aesar, 7761-88-8). Iodine was purchased from Tianli (Tianli, AR/250 g) in powder form. And gentamicin of a clinical grade was obtained from Xi'an 1st Hospital in the specification of 60000 units/mL. Dosages of the antibiotics used in the experiments are listed in Table 1 together with the carrier material EVOH. Staphylococcus aureus, one of the main pathogenic bacteria found on both animal and human wound surfaces [20, 21], was chosen to test the antibacterial ability of the nanofibers.

2.2. Experimental Procedure for Electrospinning of Nanofibers. The electrospinning system (Figure 1) used for electrospinning is an improved in-house platform developed in the previous study [18, 22]. The system is composed of a high voltage power supply with a low current output (Spellman CZE1000R, 0–30 kV, maximum 0.1 mA), a peristaltic pump

with a feeding capacity in the range of 1.0 to 15.0 mL h⁻¹ (Masterflex, 77120-52), and a feeding tube fixed with a fine metal needle at the end. All nanofiber samples were prepared from the solution of 7.5% (w/v) EVOH. Three bacterial control agents, AgNO₃, iodine, and gentamicin, were added to EVOH solution, respectively. The solutions were then injected at the speed of 2.5 mL h⁻¹ in a space field charged to 25 kV to obtain the nanofibers through the spinning of the jet. Table 1 lists the fabrication parameters of the three fiber groups used in this study.

Fibers obtained in this study were in the diameter range from 400 nanometers to 2.2 micron meters. Scanning electron microscopy (SEM) was performed to characterize the dimension profile of the fibers obtained. Figure 2(a) shows SEM image of the nanofibers obtained. Fibers are smooth and randomly oriented, with an average diameter of 500 nm. Figure 2(b) is a transmission electron microscopy (TEM) image showing a local section of a fiber obtained from EVOH solution containing AgNO₃. Shaded dots distributed inside the nanofibers are simple Ag particles according to an EDX analysis [18].

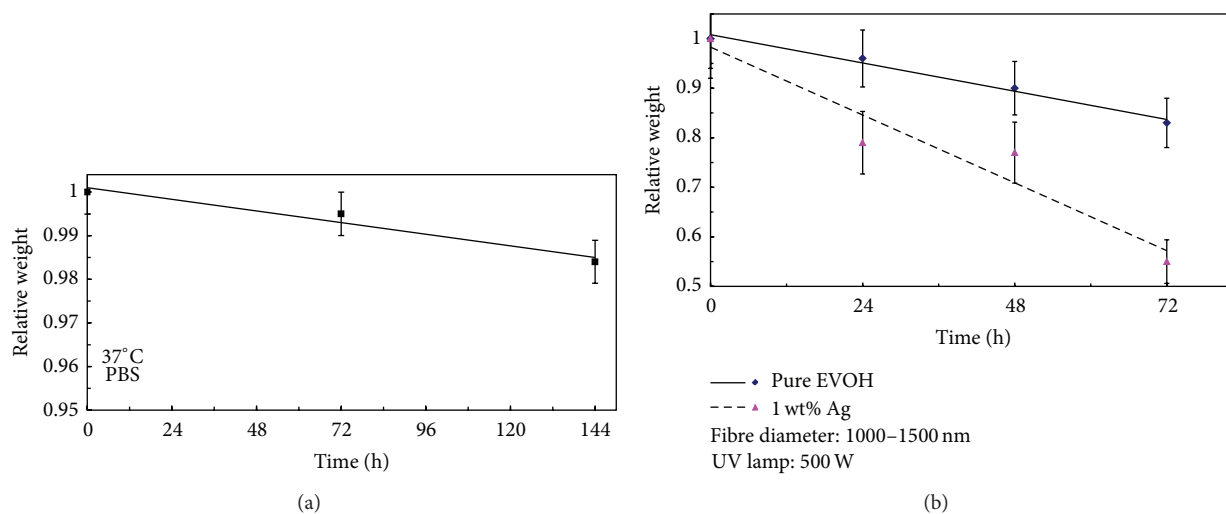


FIGURE 7: (a) Degradation of EVOH nanofiber in PBS solution. (b) UV degradation of nanofibers.

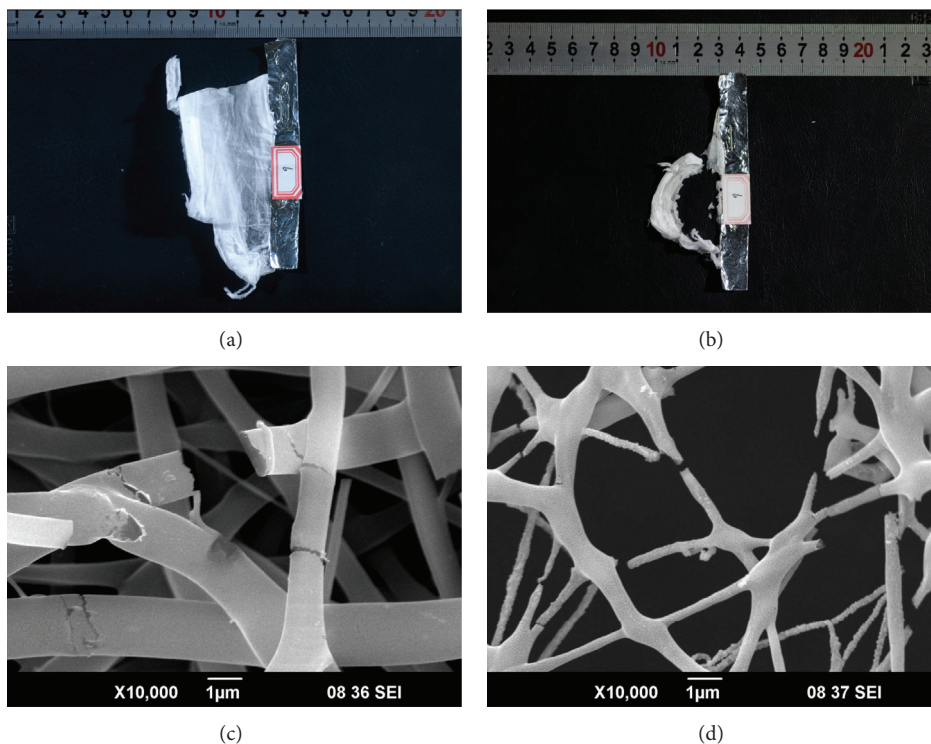


FIGURE 8: (a) Original fine fiber sample before exposure to UV light, (b) fiber sample after 72 h continuous exposure to UV light, (c) SEM image of EVOH fiber after 24 h UV exposure, and (d) SEM image of EVOH fiber after 72 h UV exposure.

TABLE 1: Materials.

Materials	Provenience	Dosage in the experiments
Poly (ethylene-co-vinyl alcohol) (EVOH)	Sigma-Aldrich (Batch number: 12822PE)	7.5% (0.75 g EVOH dissolved in 10 mL 80% 2-propanol/water)
AgNO ₃	Alfa Aesar (Alfa Aesar, 7761-88-8)	0.1 g AgNO ₃ in 10 mL EVOH solution
Iodine	Tianli (Tianli, AR/250 g)	0.1 g in 10 mL EVOH solution
Gentamicin	Xi'an 1st Hospital	60 k units in 10 mL EVOH solution

TABLE 2: Sample descriptions of EVOH nanofibers.

Sample group	Fabrication parameters	Fibre diameter range
A	7.5% solution, 15 kV, 2.5 mL/hr, 20 cm standing distance	0.4–0.9 μm
B	10% solution, 15 kV, 2.5 mL/hr, 20 cm standing distance	0.8–1.5 μm
C	12% solution, 15 kV, 2.5 mL/hr, 20 cm standing distance	1.3–2.2 μm

TABLE 3: Irradiation strength of different light sources.

Light source	Distance between the light source and the fibres/mm	Wave length/nm	Radiation strength/ $\mu\text{W cm}^{-1}$
UV lamp	50.0	240–280	3.32×10^3
Daylight lamp	2000.0	400–700	0.19
Sunlight	Ground, direct exposure	250–2500	49.9

3. Properties Related to Clinical Requirements

In this study, a few important features of the nanofibers were considered for the potential application as a wound dressing material, such as the fluid uptake ability (FUA) and the water vapour transmission rates (WVTRs), which are important characteristics in skin wound treatment. Such properties are also significant in tissue engineering where the nanofibers may also be used as scaffolding for cell growth and controlled drug release.

For wound addressing, the bacteria control ability is another key parameter, and new functionality can be developed for optimized application of antibacterial agents [23–25]. Here, only a preliminary study was carried out using the bacteriostatic loops to demonstrate the possibility of bacteria control.

The degradation of nanofibers under environmental conditions was also considered. Degradation tests in phosphate buffer solution and under ultraviolet light were carried out, providing an indication on the integrity of nanofiber materials in simulated human body conditions and accelerated environment exposures.

3.1. Fluid Uptake Ability (FUA). The fluid uptake ability (FUA) (%) of the nanofibers was calculated as a percentage of water loss per unit weight:

$$\text{FUA (\%)} = \frac{(W_s - W_d) \times 100\%}{W_s}, \quad (1)$$

where W_s is the wet weight of the nanofiber and W_d is the dry weight of the nanofiber.

A piece of dry nanofiber mat (in room condition) was first measured for its weight. It was then fully submerged into distilled water for 15 seconds. The wet mat piece was picked up and put on a fresh absorbing paper for 30 seconds, and its weight was then measured again.

The FUA experimental data of the sample Groups A, B, and C (categorized in Table 2) are given in Figure 3 compared with those of normal medical cotton gauzes, denoted as

Group D (diameters of the cotton fibers are between 0.1 and 0.2 mm). The result shows that all nanofiber groups have a higher FUA (%) than that of cotton gauze. On average, nanofibers can take 22% more water than cotton gauze per unit weight.

Among the nanofiber groups, Group C has the highest FUA at $(85.97 \pm 0.7)\%$, while Group A has the lowest $(81.66 \pm 0.8)\%$, though the difference is only 5%. Figure 3 appears to suggest that in the diameter range smaller than the micron level, a larger diameter will lead to a higher FUA. But when the fiber diameter is above the micron level, such as in cotton gauze, FUA will drop with increased diameter.

3.2. Water Vapour Transmission Rates (WVTRs). The water vapour transmission performance of the nanofibers was measured in the following approach. Beakers filled with 5 mL distilled water were covered with a nanofiber mat of the three sample groups, respectively. The covered beakers were placed in an airtight environment chamber with saturated ammonium sulfate solution. The weight of the beakers was measured once every 24 hours over a period of 120 hours (5 days). The difference of the measured weight was divided by the cross-section area of the beaker. The water vapour transmission rates, WVTRs ($\text{g}\cdot\text{m}^{-2}$ per day), were calculated as the weight loss of water (gram) per evaporation exposure area (m^2) per day [26].

Measured WVTR of the nanofibers is given in Figure 4, showing that WVTR increases rapidly in Day 1. The 24-hour measurement span means that there is only one data point at the end of Day 1; thus, higher points which could occur within Day 1 may have been missed. However, the trend is captured. WVTR drops slightly from Day 1 and remains virtually constant from Day 2 onwards with the values kept in the range of 900 to 1000 $\text{g}\cdot\text{m}^{-2}$ per day. There is virtually no difference between Groups A, B, and C. Group A has a slightly higher WVTR, close to that of Group D, which shows the highest WVTR constantly, though marginally.

3.3. Antibacterial Tests. To evaluate the clinical related performance of the nanofibers, antibacterial tests were carried out using culture dishes. Clear dishes were first covered with LB culture mixed with *Staphylococcus aureus* (OD600 value: 1.1). One circular pad of 6 mm diameter, cut from each of the nanofiber samples containing Ag, iodine, and gentamicin, respectively, and from pure EVOH nanofibers with no antibacterial agent as the control group, was placed at equal distance in a culture dish. The quantity of the antibiotic agents contained in the nanofibers is, before electrospinning, as follows:

gentamicin, 60 k units in 10 mL solution;

Ag nanoparticle, 0.1 g AgNO₃ in 10 mL solution, and iodine, 0.1 g in 10 mL solution.

The culture dishes hosting the nanofiber pads were then placed on a disk in a nursery box which was maintained at 37°C. The disk was rotated continually inside the nursery box at a constant rotational speed of frequency of 1 Hz.

Figure 5 illustrates one of the culture dishes after 6 hours of incubation at 37°C, showing the four pads of samples with pure EVOH fibres containing no antibacterial agent marked as (a), and fibres containing gentamicin (b), Ag nanoparticle (c), and iodine (d). While the disk is covered by cultured *Staphylococcus aureus* seen in a foggy colour, the clear shaded rings surrounding the pads (red circles indicate the original pad size) are bacteriostatic loops where *Staphylococcus aureus* had been eliminated. A bigger outer ring diameter indicates stronger antibacterial effectiveness. It can be clearly seen in Figure 5 that Ag (c) has the biggest bacteriostatic loop while pure EVOH (a) and gentamicin (b) show limited effectiveness.

Figure 6(a) shows the measured outer diameters of the bacteriostatic loops. Nanofibers containing Ag nanoparticles demonstrate the strongest germ killing capacity and the outer diameter of its bacteriostatic loop is 58, 174, and 420%, which is bigger than the one of iodine, gentamicin, and pure EVOH, respectively. Consequently, only the influence of the Ag density was further examined. Nanofibers using solutions dissolved with different weight of AgNO₃ were produced and used for antibacterial tests. Figure 6(b) illustrates the outer diameter of the bacteriostatic ring as a function of the weight of Ag content in the nanofibers, measured in grams per milliliter. The test set-up remained the same except the culture time which was increased to 12 hours for this Ag effectiveness study. As seen in Figure 6(b), the bacteriostatic effectiveness shows approximately a power law relation to the Ag density in the nanofibers.

4. Degradation of EVOH Nanofibers

The integrity of nanofibers under environmental elements during production, storage, transportation, and usage is an important factor for the application. To investigate this, hydrolysis tests and ultraviolet light exposure tests were performed. For the hydrolysis tests, nanofiber samples were submerged in phosphate buffer solution (PBS) of pH value of 7.2 and kept at 37°C. The test was aimed to evaluate

time-based stability of the material in simulated *in vivo* conditions. The samples were weighted every 72 hours with a bioelectronic balance after 30 mins of drying at 37°C.

The ultraviolet exposure tests were performed in a photochemical reaction chamber. The irradiation strength of the light at 240–280 nm wavelengths was $3.23 \times 10^3 \mu\text{W cm}^{-2}$ as measured with a UV radiometer at the point where the samples were placed. The samples were weighted and photographed every 24 hours of exposure.

Hydrolysis *in vitro* shows that the nanofibers hardly decompose in the simulated body environment. As shown in Figure 7(a), only 2% weight change of the samples was recorded after being submerged in PBS solution for 168 hours (7 days). Also, little degradation was observed. These indicate good integrity of the nanofibers for long term use in the human body condition, an importance requirement on dressing materials.

In contrast to the hydrolysis tests, significant weight loss and fiber break-up were observed during the UV exposure tests (see Table 3 for comparable light exposures). Figure 7(b) shows the weight variation of the nanofibers with and without Ag nanoparticles versus the UV exposure hours. It can be seen that the nanofibers degrade approximately linearly in terms of the exposure duration. And those containing Ag nanoparticles degrade faster with more than 40% weight reduction over 72 hours (3 days) of exposure, twice the weight loss in pure EVOH nanofibers.

In the format of the degradation, nanofibers show strong signs of disintegration with shrinkage and break-down into pieces, as shown in Figure 8. Figures 8(a) and 8(b) provide a graphic revelation on nanofibers' texture deterioration under UV exposure. The relatively short duration for almost complete structural destruction just over 72 hours indicates a high sensitivity to UV lights. SEM images (Figures 8(c) and 8(d)) show nanofibers breaking up and thinning out, resulting in fabric structural destruction.

To further study the light sensitivity of the nanofibers, the effects of exposure to different light sources were compared. The irradiations of an UV lamp, a daylight lamp, and direct sunlight were compared. In Table 2, the irradiation strength of an UV lamp is 66 times stronger than that of the direct sunlight exposure. Results of degradation under UV lamp indicate that the nanofibers show signs of deteriorated structures after 24 hours and are largely destroyed after 72 hours. This is equivalent to about half a year of exposure to direct sunlight.

The high sensitivity in EVOH nanofibers degradation under UV exposure leads to two interesting features. Firstly, the UV light may be used as a mechanism to speed up the release of Ag nanoparticles and possibly other drugs, through the material break-up of the nanofibers when they are used as a carrier. Secondly, the rapid texture deterioration under UV lighting also means there would be implications in using the EVOH nanofibers as wound dressing or bandage for a prolonged period, where some UV shielding might be required.

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5. Conclusions

The EVOH nanofibers were obtained in this study from an electrospinning process. Experimental studies carried out in the lab on some of the performance related to clinical applications have led to the following conclusions.

- (1) The electrospinning process does not inactivate the antibacterial ability of encapsulated agents.
- (2) Nanofibers show a good fluid uptake ability (FUA), taking 22% more water than cotton gauze per unit weight.
- (3) Nanofibers' water vapour transmission rates (WVTRs) are comparable to those of cotton gauze.
- (4) Nanofibers containing silver particles demonstrate a superior antibacterial capacity than those containing gentamicin and iodine.
- (5) The rapid degradation of nanofibers indicates that shielding of UV exposure might be needed for prolonged usage, but the feature may be used for controlled drug release.
- (6) Nanofibers are found to be stable *in vitro* condition, thus potentially a good candidate for wound dressing applications.

Based on the outcome of the study, we can draw the conclusion that EVOH nanofibers are potentially a promising candidate for skin wound dressing. In particular, the drug carrying and slow release functions can be further explored for optimal usage. As the study was based on a small quantity of samples, results may be affected by random factors, though care was taken to eliminate noticeable uncertainties. However, more tests are needed on a large quantity and in a broader scope and wider range of parameters. These will be addressed in the continuity of this research programme.

Nomenclature

EVOH:	Poly (ethylene-co-vinyl alcohol)
FUA:	Fluid uptake ability
PBS:	Phosphate buffer solution
SEM:	Scanning electron microscopy
TEM:	Transmission electron microscopy
UV:	Ultraviolet
WVTR:	Water vapour transmission rates (WVTRs)
W_s :	Wet weight of the nanofiber
W_d :	The dry weight of the nanofiber.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Anhui Wang and Chao Xu contributed equally to this paper.

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